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Emerging Methods for Biological Control of Barley Diseases Including the Role of Endophytes

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Abstract

Barley is an important crop worldwide with production largely used for animal feed and alcoholic beverages. Diseases are a major limiting factor to its production. These have, up until recently, been controlled by agrochemicals. However, legislation on the use of agrochemicals, especially within the European Union, is being tightened and there is growing interest in integrated pest management. This means that there is an increasing focus on controlling diseases using biological control. Living microorganisms that are applied as biological control agents (BCAs) to either soil, seed or leaves can have difficulty in persisting. Therefore, the focus of this review is on endophytes, which are microorganisms that live inside the plant without causing symptoms of disease and have the potential of staying protected as well as being beneficial to the plant and effective against multiple diseases. In this review, we discuss the different approaches for finding and testing beneficial endophytes and for determining the endophyte host range. Furthermore, we undertook a literature search to summarise previous studies that have investigated the use of endophytes as well as BCAs against barley diseases.

5.1 Important Diseases of Barley in Northern Europe

Barley is the fourth largest cereal crop in the world with a global production of more than 141 million tonnes (FAOSTAT, 2018). Barley is used primarily as animal

feed (55–60% of total production), for seeds (5%) and to a much lesser extent for human consumption (2–3%). In addition, malting barley is used for the production of alcoholic beverages (30–40%) (Ullrich, 2011). In cereals, it is estimated that pre-harvest losses, due to disease, weeds and environmental stresses, can reach up to approximately 35% of the yield (Akar *et al.*, 2004). Losses due to disease are particularly damaging, and there are many important barley diseases worldwide. Some of the most important in Northern Europe are: powdery mildew (*Blumeria graminis* f. sp. *hordei*), leaf blotch or scald (*Rhynchosporium graminicola*), rusts such as brown rust (*Puccinia hordei*) and yellow rust (*P. striiformis* f. sp. *hordei*), net blotch (*Pyrenophora teres*), Ramularia leaf spot (*Ramularia collo-cygni*) and barley yellow dwarf (*BYDV*) (Oldach, pers. comm.; Walters *et al.*, 2012), which all cause symptoms on leaves (Mathre, 1982; Videira *et al.*, 2016). In addition, Fusarium head blight (*Fusarium* spp.) affects the malting quality and yield of malt when producing beer (Oliveira *et al.*, 2012; Nielsen *et al.*, 2014) with symptoms occurring in the heads (Mathre, 1982). It is expected that the use of some agrochemicals will be banned or restricted in the future (Oldach, pers. comm.), which means that the reliance on chemical inputs will need to be reduced. Furthermore, organic agriculture has increased by almost 20% a year globally (Nandwani and Nwosisi, 2016) and farmers would also benefit from an alternative non-synthetic solution.

A European Union regulation (Directive 2009/128/EC; European Parliament, 2009) has already been implemented, which is encouraging the sustainable use of pesticides and promoting integrated pest management (Department of Agriculture, 2013). Integrated pest management is defined by ENDURE (2008) as 'a sustainable approach to managing pests by combining biological, cultural and chemical tools in a way that minimises economic, environmental and health risks'. The use of biopesticides is promoted as an environmentally friendly alternative to synthetic pesticides. Within Europe, the term 'biopesticides' is often used to cover a range of products that can be used to protect crops from diseases, pests and weeds. The products can be divided into two subgroups that either rely on the use of (1) living organisms or (2) naturally occurring compounds, including extracts from plants and microorganisms as well as volatiles (Balog *et al.*, 2017). There are a limited number of registered biopesticide products in Europe and they are based on bacteria, fungi or viruses (Kabaluk *et al.*, 2010; Balog *et al.*, 2017). In most cases, the control agent is a different species to the plant pathogen. However, in some cases weak strains or non-pathogenic isolates of the same pathogen species are used (Punja, 1997; Kabaluk *et al.*, 2010). Within the scientific community, biological control has most often been defined as the use of living organisms for crop protection (Pal and Gardener, 2006) and here the term biological control is more precisely defined as the control of plant diseases by microorganisms. Biological control of plant diseases can work through one or a combination of four general mechanisms which are (1) parasitism, (2) antibiosis, (3) competition and (4) induction of host resistance

(Guetsky *et al.*, 2002; Alabouvette *et al.*, 2006). Integrated control of foliar barley diseases is reviewed by Walters *et al.* (2012). However, they do not include the use of biological control.

5.1.1 Endophytes Used for Biological Control of Plant Diseases

The interest in using microorganisms as biological control agents of plant diseases is increasing (Broadfoot, 2016), especially for diseases that are otherwise difficult to control (Walters, 2009). The plant microbiome consists of epiphytes and endophytes (Lindow and Brandl, 2003; Müller *et al.*, 2016). Epiphytes are the microorganisms that live on plant surfaces and they can be further divided into organisms that inhabit the rhizosphere, the phyllosphere (Müller *et al.*, 2016) and the spermosphere (Lindsey *et al.*, 2017). The term 'endophyte' was first used by Anton de Bary in 1884. He described an endophyte as a parasite living inside its host's organ (de Bary, 1884). The definition has since then been broadened and endophytes are generally defined as microorganisms living inside plants without causing symptoms of disease (Wilson, 1995). Some endophytes have been shown to provide plants with benefits such as drought tolerance (Naveed *et al.*, 2014), heat tolerance (Hubbard *et al.*, 2014), improved mineral nutrition (Murphy *et al.*, 2015a), salt-stress tolerance (Rodriguez *et al.*, 2008) and protection against disease (Maciá-Vicente *et al.*, 2009). While endophytes are not the only biocontrol approach to have received attention in recent years, they compare favourably to other microorganisms which may have difficulty persisting and/or remaining competent when they are applied to the leaves, the seeds or the soil (Walker *et al.*, 2002; Ting *et al.*, 2009; Buddrus-Schiemann *et al.*, 2010). Thus, the use of endophytes may keep the biological control agents (BCAs) protected within the plant (Eevers *et al.*, 2015) and provides the possibility of control against several stresses without losing efficacy over the growing season (Wilkinson *et al.*, 2000). In our research, focus is put on generalist endophytes, which can be transferred from crop wild relatives (CWRs) and promising results have been obtained in barley (A. K. Høyer, unpublished results). Thus, this review will emphasise the targeted search for plant protecting endophytes as well as previous studies of biocontrol in barley.

5.1.2 Endophyte Host Range and the Targeted Search for Beneficial Endophytes

All plants in natural habitats are believed to harbour endophytes (Aly *et al.*, 2011) and they can be tissue-type specific or systemic (Zabalgogeazcoa, 2008). The life cycles of the majority of endophytes are not completely understood, but it is clear that some endophytes do not remain exclusively within the plant throughout their whole life cycle, which means that they can potentially be latent pathogens (Comby *et al.*, 2016) or latent saprotrophs, or can represent early colonisation by rhizobia or mycorrhizal fungi (Porras-Alfaro and Bayman, 2011). The diversity of different

taxonomic groups of endophytes that has been elucidated recently has been summarised in the meta-analysis by Hardoim *et al.* (2015). The most frequently reported sequences of prokaryotic endophytes were from Proteobacteria (54%), Actinobacteria (almost 20%) and Bacilli (15%), whereas eukaryotic sequences were mostly from Glomeromycota (40%, arbuscular mycorrhizal fungi), Ascomycota (almost 31%, with the subordinate class Dothideomycetes accounting for 15%) and Basidiomycota (20%, with Agaricomycetes accounting for 18%).

Many factors have been shown to influence the endophyte community composition and one of the important factors is host plant species (Nissinen *et al.*, 2012; Wearn *et al.*, 2012). Nissinen *et al.* (2012) showed that several bacterial genera were tightly associated with particular arcto-alpine plant species (*Oxyria digyna*, *Diapensia lapponica* and *Juncus trifidus*). In total, they identified 58 different bacterial genera. Of the major bacterial genera, five were exclusively associated with *J. trifidus* (*Acido Gp1*, *Arthrobacter*, *Knoellia*, *Paenibacillus*, *Paracoccus* and *Rhodanobacter*), four were specific to *O. digyna* (*Agreia*, *Ancylobacter*, *Rhizobium* and *Rhodococcus*) and one was exclusively associated with *D. lapponica* (*Pedobacter*). However, some groups of endophytes are generalist and are able to colonise plants of unrelated taxonomic identity. Interestingly, all three plant species were colonised by *Burkholderia*, *Mucilaginibacter*, *Nocardioides* and *Sphingomonas*. Wearn *et al.* (2012) explained that part of the fungal communities of grassland forbs (*Cirsium arvense*, *Plantago lanceolata* and *Rumex acetosa*) were host-plant specific. Thus, 48% of the fungal community belonging to *C. arvense* was generalist endophytes, with 58% and 72% generalists for *P. lanceolata* and *R. acetosa*, respectively (Wearn *et al.*, 2012). In grasses, generalist endophytes are, for instance, found in the groups of clavicipitaceous endophytes and dark septate endophytes (DSEs) (Clay, 1990; Jumpponen and Trappe, 1998; Mandyam *et al.*, 2010). Known generalists of clavicipitaceous endophytes in temperate grasses are *Epichloë coenophiala* and other *Epichloë* ssp., which infect grasses in the subfamily Pooideae (Hodkinson, 2018) and *Atkinsonella* spp. which infects *Danthonia* spp. and *Stipa* spp. (Clay, 1990). For generalists within the DSEs, Mandyam *et al.* (2010) showed that the roots of four C_4 grasses (*Andropogon gerardii*, *Sorghastrum nutans*, *Schizachyrium scoparium* and *Panicum virgatum*) normally had two DSEs in common, i.e. *Periconia macrospinosa* and *Microdochium* sp..

Several approaches have been explored to isolate potential endophytes that confer protection against diseases. Most studies have cultured endophytes from healthy looking plants that live in an environment that has a particular disease stress. In this case, it is hypothesised that the endophytes contribute to plant health and that they are able to relieve the stress (Araujo *et al.*, 2002). An alternative strategy has been suggested by Ellis (2017), who proposed looking for biocontrol agents in diseased tissue because organisms can persist in a pathogen-infected tissue and hence potentially act as control agents. Although this may appear as counter-intuitive,

Ellis (2017) gives an example of control of crown gall in stone fruit and, furthermore, Köhl *et al.* (2009) found antagonists supressing apple scab using this approach.

There are different approaches when it comes to both selecting target plant species as sources of beneficial endophytes and selecting a plant species to test the biological control properties. Most studies have isolated endophytes from a crop species and then tested the biological control effects in the original crop (Kirk and Deacon, 1987; Coombs *et al.*, 2004; Silva *et al.*, 2012). Some studies isolated endophytes from related taxonomic groups of a crop species and tested the effect in the crop (Maciá-Vicente *et al.*, 2008). CWRs are valuable resources in crop breeding programmes and have been used to transfer disease resistance (Zeng *et al.*, 2013; Brar and Hucl, 2017; Fedak *et al.*, 2017). Likewise, CWRs can be a unique source of potential biocontrol agents (Maciá-Vicente *et al.* 2008; Murphy *et al.* 2015b). CWRs will have their own microbiome and, although not adequately tested, could be expected to host some endophytes not ordinarily present in the crop species. Due to the close taxonomic affinity to the crop plants, they could also be expected to be more compatible to the target species than endophytes isolated from an unrelated species (Murphy *et al.*, 2018). In addition, some endophytes are isolated from an unrelated plant and then tested on crops. For example, *Serendipita indica* (formerly *Piriformospora indica*; Weiß *et al.*, 2016) is a basidiomycete endophyte that has been tested on many different crops, which are not closely related to the original host (Kumar *et al.*, 2009; Harrach *et al.*, 2013; Rabiey *et al.*, 2015; Wang *et al.*, 2015). *Serendipita indica* was isolated from the rhizosphere of two woody shrubs, *Prosopis juliflora* and *Zizyphus nummularia*, in desert soils of Rajasthan in India (Varma *et al.*, 2012). This fungus has been tested as a BCA in several crop species including wheat (*Triticum aestivum*; Serfling *et al.*, 2007; Rabiey *et al.*, 2015), barley (*Hordeum vulgare;* Harrach *et al.*, 2013), maize (*Zea mays*; Kumar *et al.*, 2009) and tomato (*Solanum lycopersicum;* Roylawar *et al.*, 2015; Wang *et al.*, 2015).

5.2 Previous Studies of Endophytes and Other BCAs Controlling Barley Diseases

An extensive literature survey, conducted here, revealed a total of 8 studies reporting the control of barley diseases by endophytes and 21 studies reporting control by other BCAs (Table 5.1). Only studies using living microorganisms were included in the review. Eight different fungal endophyte species were tested in the endophyte studies and *Serendipita indica* was tested in four of the investigations. In the studies reporting control by BCAs, several different organisms were used, with the majority using fungi. *Pseudomonas* spp. strains were widely used followed by *Trichoderma* spp. and *Clonostachys rosea*. Although not tested as endophytes in the investigations reviewed here, these commonly used organisms have often been widely isolated as

Table 5.1 Overview of the investigations on biological control of barley diseases.

The first part of the table lists studies using endophytes and the second part lists studies using other types of biological control agents. The investigations are organised first according to pathogen, second according to efficiency of disease control and third according to year of publication. The investigations are evaluated on a scale where '0' is given to reports where there was no disease control, '+' is given to reports were disease control efficiency of 0.01–33% are described, '++' are reports of 33–66% efficiency and '+++' are reports of 66–100% efficiency. The minus symbol indicates that the investigation did not look into disease control or the mechanism of control. Evalution of disease control and mechanism is reported according to the test system. Names of organisms are given according to Species Fungorum (www.speciesfungorum.org) and Catalogue of Life. Footnotes are listed at the end of the table.

Pathogen	Endophyte	Endophyte origin	Test system	Disease control efficiency	Suggested mechanism of control	Author
Bipolaris sorokiniana	*Chaetomium globosum*	Barley leaves	Dual culture	+[1]/++[2,3]	Antibiosis and competition[4]	Moya *et al.*, 2016
Pyrenophora teres					Competition and mycoparasitism[5]	
Bipolaris sorokiniana	*Serendipita indica*	Roots of woody shrubs	Detached-leaf-segment assay	++[6]	Systemic induction of resistance associated	Waller *et al.*, 2005
Blumeria graminis f. sp. *hordei*			Pot trials	0[7]	with elevated	
Fusarium culmorum					antioxidative capacity	
Blumeria graminis f. sp. *hordei*	*Dichotomopilus funicola*	Tomato roots	*In vitro* spore germination test	++	Antibiosis	Vilich *et al.*, 1998
	Chaetomium globosum	Laboratory strain[8]	Pot trials	+/++[9]	-	
Fusarium culmorum	*Serendipita indica*	Roots of woody shrubs	Pot trials with soil	+	Elevated antioxidative capacity	Harrach *et al.*, 2013

Fusarium graminearum	*Serendipita indica*	Roots of woody shrubs	Dual culture	**0**[10]	Not antibiosis	Deshmukh and Kogel, 2007
			Pot trials with soil	**+++**	Not induced resistance	
Fusarium graminearum	*Serendipita indica*	Roots of woody shrubs	Pot trial with soil	**0**[11]	Growth acceleration	Achatz *et al.*, 2010
Gaeumannomyces tritici	*Acremonium furcatum*	24 plant species	Dual plate bioassay	**+**	-	Maciá-Vicente *et al.*, 2008
	Dactylaria sp.		Tube with vermiculite	**+++**	-	
	Fusarium equiseti					
	Phoma herbarum					
Gaeumannomyces tritici	*Fusarium equiseti*	*Corynephorus canescens* and *Lygeum spartum*	Tube with vermiculite	**++**	-	Maciá-Vicente *et al.*, 2009
	Metacordyceps chlamydosporia	*Heterodera avenae* infected eggs and *Meloidogyne* sp.				
Pathogen	**BCA**	**BCA origin**	**Test system**	**Disease control efficiency**	**Suggested mechanism of control**	**Author**
Bipolaris sorokiniana	*Clonostachys rosea*	Barley roots	Pot trial with sand	**++/+++**[12]	-	Jensen *et al.*, 2002

(*continued*)

Table 5.1 (Cont.)

Pathogen	BCA	BCA origin	Test system	Disease control efficiency	Suggested mechanism of control	Author
Bipolaris sorokiniana *Blumeria graminis f. sp. hordei* *Pyrenophora teres* *Rhynchosporium graminicola*	*Bipolaris maydis* *Parastagonospora nodorum*	Maize Wheat	Pot trial with soil	+[13]/++[14]/+++[15]	-	Jørgensen *et al.*, 1996
Bipolaris sorokiniana *Blumeria graminis* f. sp. *hordei* *Pyrenophora teres* *Rhynchosporium graminicola*	*Clonostachys rosea*	Barley roots	Pot trial with soil	**0**[16]/++[17]/+++[18]	Direct inhibition. Reduced conidial germination and appressorium formation[19]	Jensen *et al.*, 2016
Bipolaris sorokiniana *Microdochium nivale* *Pyrenophora graminea*	*Pseudomonas chlororaphis*	Soil	Field experiments	+[20]/++[21]/+++[22]	-	Johnsson *et al.*, 1998

Pyrenophora teres						
Ustilago hordei						
Ustilago nuda						
Bipolaris sorokiniana	*Acremonium sp.*	Barley seed	Pot trial with sand	+/++	-	Knudsen *et al.*, 1995
	Chaetomium sp.	Barley seed	Pot trial with soil	+/++	-	
	Clonostachys rosea	Organic soil	Field experiments	+/++	-	
	Fusarium roseum	Conventional soil				
	Humicola sp.	Conventional hay				
	Microdochium bolleyi	Organic hay				
	Trichoderma sp.	Roots				
Bipolaris sorokiniana	*Microdochium bolleyi*	Grassland	Cellulose filter paper rolls	+	Induced resistance	Liljeroth and Bryngelsson, 2002
			Pot trials with soil	++		
			Field experiment	-[23]		
Bipolaris sorokiniana	*Microdochium bolleyi*	Wheat coleoptile	Field experiments	+	-	Duczek, 1997
Blumeria graminis f. sp. *hordei*	*Bacillus thuringiensis* (14 isolates)	Faecal samples from mammalian species	Pot trial	+/+++	-	Choi *et al.*, 2007

(*continued*)

Table 5.1 (Cont.)

Pathogen	BCA	BCA origin	Test system	Disease control efficiency	Suggested mechanism of control	Author
Fusarium culmorum	*Clonostachys rosea*	Barley roots	Pot trial with sand	++/+++	-	Jensen *et al.*, 2000
			Field experiments	++/+++		
Fusarium culmorum	*Actinomycetes* (133 strains)	Saharan soil	Streak method	-[24]	-	Yekkour *et al.*, 2012
			Petri dishes with filter paper	+/++/+++	-	
Fusarium culmorum	*Acinetobacter* sp.	Cereal plant and soil	Dual culture	+/++/+++	Not antibiosis	Khan *et al.*, 2006
Fusarium graminearum	*Chryseobacterium* sp.		Pot trial with soil	+/++/+++	Induced resistance[25]	
Fusarium poae	*Pseudomonas fluorescens*					
	Pseudomonas frederiksbergensis					
	Pseudomonas sp.					
	unidentified isolate					
Fusarium culmorum	*Acinetobacter* sp.	Cereal plant and soil	Pot trial with soil	+/++[26]	-	Khan and Doohan, 2008a

Fusarium graminearum	*Chryseobacterium* sp.		Field experiment	++	-	
Fusarium poae	*Pseudomonas fluorescens*					
	Pseudomonas frederiksbergensis					
	Pseudomonas sp.					
Fusarium culmorum	*Pseudomonas fluorescens*	Barley rhizosphere soil	Pot trial with soil	++	-	Khan and Doohan, 2008b
			Field experiment	++	-	
Gaeumannomyces tritici	*Metacordyceps chlamydosporia*	Nematophagous fungi	Petri dish experiment	++/+++	-	Monfort *et al.*, 2005
	Metapochonia rubescens		Tube with vermiculite	+	-	
	Lecanicillium lecanii					
Pyrenophora graminea	*Streptomyces* ssp. (5 strains)	-	Pot trial with soil	+/++/+++	-	Koch *et al.*, 2006
Pyrenophora teres	*Trichoderma* spp. (5 strains)	-				
Pyrenophora teres	Actinomycete	Soil and straw	*In vitro* straw assay	+++	-	Mostafa, 1993
	Albifimbria verrucaria		Tube with cotton	++/+++	-	

(*continued*)

Table 5.1 (Cont.)

Pathogen	BCA	BCA origin	Test system	Disease control efficiency	Suggested mechanism of control	Author
	Trichoderma viride		Pot trial[27]	+++	-	
	Trichoderma pseudokoningii					
	Trichoderma sp.					
Pyrenophora teres	400 bacterial isolates	Roots of wild and	Pot trial with soil	+/++/+++	-	Hökeberg *et al.*, 1997
	Pseudomonas isolates	cultivated plants	Field experiment	+++	-	
Pyrenophora teres	*Micromonospora*	Barley straw	*In vitro* straw assay	+/++/+++	-	Ali-Hoimoud *et al.*, 1993
	Trichoderma koningii					
	Trichoderma pseudokoningii					
	Trichoderma viride					
	Five unidentified fungi					
Pyrenophora teres	Nine fungal antagonists[28]	Soil and roots of barley sprouts	Pot trial with sand	+/++/+++	-	Abrahamsen, 1992
Pyrenophora teres	*Bipolaris maydis*	Maize	Pot trial with soil	+/+++	Induced resistance	Jørgensen *et al.*, 1998
	Parastagonospora nodorum	Wheat				

Pseudomonas syringae	*Pantoea agglomerans*[29]	Barley seeds	Pot trial[30]	++/+++	-[31]	Braun-Kiewnick *et al.*, 2000
			Field experiments	+/++/+++		

1 For *Bipolaris sorokiniana*
2 For *Pyrenophora teres*
3 Some treatments gave reductions of 0–33% / others gave reductions of 33–66% / while others gave reductions of 66–100%
4 For *Bipolaris sorokiniana*
5 For *Pyrenophora teres*
6 For *Blumeria graminis* f. sp. *hordei*
7 Methods of evaluation was insufficient
8 Perhaps it originated from twigs of *Quercus* sp., however it is unclear
9 For *Chaetomium globosum*
10 No inhibition zone was formed
11 Methods of evaluation were insufficient
12 Dependent on storage conditions
13 For *Bipolaris sorokiniana* and *Blumeria graminis* f. sp. *hordei.* Results are cultivar dependent
14 For *Bipolaris sorokiniana*, *Pyrenophora teres* and *Rhynchosporium graminicola.* Results are cultivar dependent
15 For *Rhynchosporium graminicola.* Results are cultivar dependent
16 For *Blumeria graminis* f. sp. *hordei*
17 For *Bipolaris sorokiniana* and *Pyrenophora teres*
18 For *Rhynchosporium graminicola*
19 For *Bipolaris sorokiniana*
20 Against *Ustilago nuda*
21 Against *Bipolaris sorokiniana* and *Microdochium nivale*
22 Against *Pyrenophora graminea*, *Pyrenophora teres*, *Ustilago hordei* and *Ustilago nuda*
23 No disease symptoms occurred before ear emergence
24 No control treatment – difficult to estimate the disease reduction. Antagonists were tested against several *Fusarium* ssp., *Aspergillus* ssp. and *Penicillium* ssp. at this step
25 Results are from wheat not barley
26 Dependent on timing of BCA application
27 Substrate not mentioned
28 Identity not mentioned
29 Several strains
30 Substrate not mentioned
31 Investigated but mentioned in a different paper

endophytes (Evans *et al.*, 2003; Høyer *et al.*, 2016; Mercado-Blanco *et al.*, 2016). All the studies collectively focus on a limited number of pathogens and they include the leaf pathogens *Bipolaris sorokiniana*, *Blumeria graminis* f. sp. *hordei*, *Pseudomonas syringae*, *Pyrenophora graminea*, *Pyrenophora teres*, *Rhynchosporium graminicola*, *Ustilago hordei*, *U. nuda*, as well as the soil pathogens *Bipolaris sorokiniana*, *Fusarium culmorum*, *F. graminearum*, *F. poae*, *Gaeumannomyces tritici* and *Microdochium nivale*.

The rationale behind the selection of host plant species as a source of biological control agents has often not been described sufficiently well in the studies. The endophytes were sourced from barley (Moya *et al.*, 2016), grasses including *Ammophila arenaria* ssp. *australis*, *Corynephorus canescens* and *Lygeum spartum* (Maciá-Vicente *et al.*, 2008, 2009) and unrelated plant species (Vilich *et al.*, 1998; Waller *et al.*, 2005; Maciá-Vicente *et al.*, 2008; Deshmukh and Kogel, 2007; Achatz *et al.*, 2010; Harrach *et al.*, 2013). Among the studies using other BCAs, one study did not describe where the BCA originated from (Koch *et al.*, 2006). Seven out of 21 studies used antagonists that originated from barley (Abrahamsen, 1992; Ali-Hoimoud *et al.*, 1993; Knudsen *et al.*, 1995; Braun-Kiewnick *et al.*, 2000; Jensen *et al.*, 2000, 2002, 2016), five studies used BCAs from other cereals or what was termed 'straw' (Mostafa, 1993; Knudsen *et al.*, 1995; Duczek, 1997; Jørgensen *et al.*, 1996, 1998) and two studies used antagonists originating from unspecified grasses (Hökeberg *et al.*, 1997; Liljeroth and Bryngelsson, 2002). One study used BCAs from unrelated plant species (Hökeberg *et al.*, 1997) and seven used samples from soil (Mostafa, 1993; Knudsen *et al.*, 1995; Johnsson *et al.*, 1998; Khan *et al.*, 2006; Khan and Doohan, 2008a, 2008b; Yekkour *et al.*, 2012). Two studies used nematophagous fungi (Monfort *et al.*, 2005; Maciá-Vicente *et al.*, 2009), one used fungi isolated from insects (Maciá-Vicente *et al.*, 2009) and one study found their control agents in mammalian faeces (Choi *et al.*, 2007).

Four different reasons for working with a specific endophyte species were given (Table 5.1) including getting good results from a preliminary *in vitro* study (Maciá-Vicente *et al.*, 2008), the fact that the endophyte belonged to a genus which is known for the production of secondary metabolites (Vilich *et al.*, 1998), previous success with the organism within the research group (Maciá-Vicente *et al.*, 2009; Achatz *et al.*, 2010) or a literature review (Deshmukh and Kogel, 2007; Achatz *et al.*, 2010; Harrach *et al.*, 2013; Moya *et al.*, 2016). The choice of BCA species in the other studies was often explained by the fact that the organisms had been used successfully in previous studies by the same authors or research group (Knudsen *et al.*, 1995; Jensen *et al.*, 2000, 2002, Koch *et al.*, 2006; Khan and Doohan, 2008b; Jensen *et al.*, 2016). However, a short literature review of the abilities of the BCAs in different crops or against specific diseases was more common (Mostafa, 1993; Duczek, 1997; Braun-Kiewnick *et al.*, 2000; Liljeroth and Bryngelsson, 2002; Monfort *et al.*, 2005; Choi *et al.*, 2007; Khan *et al.*, 2006; Khan and Doohan, 2008a; Yekkour *et al.*, 2012).

Much rarer reasoning was that the organisms were screened *in planta* in the actual study (Hökeberg *et al.*, 1997; Johnsson *et al.*, 1998), or were non-pathogens of barley (Jørgensen *et al.*, 1996, 1998). Sometimes no reason was given (Abrahamsen, 1992; Ali-Hoimoud *et al.*, 1993).

5.2.1 Experimental Test Systems

There is increasing financial expense in progressing from *in vitro* studies, to pot trials, to field experiments. There is, however, also an increase in the value of the knowledge produced, at least if the aim is to reduce disease pressure in the field. *In vitro* studies are, in general, controversial because there is often not a good correlation between *in vitro* results and results obtained from more complex growth systems (Renwick *et al.*, 1991; Fravel, 1988; Khan *et al.*, 2006; Deshmukh and Kogel, 2007).

In two of the biocontrol investigations (Table 5.1), long-term field trials were used (Duczek, 1997; Johnsson *et al.*, 1998). However, the most common experimental test system is pot trials (Abrahamsen, 1992; Jørgensen *et al.*, 1996, 1998; Jensen *et al.*, 2002; Koch *et al.*, 2006; Choi *et al.*, 2007; Achatz *et al.*, 2010; Harrach *et al.*, 2013; Jensen *et al.*, 2016), combined with *in vitro* testing (Mostafa, 1993; Vilich *et al.*, 1998; Khan *et al.*, 2006; Waller *et al.*, 2005; Deshmukh and Kogel, 2007) or followed by field experiments (Knudsen *et al.*, 1995; Hökeberg *et al.*, 1997; Jensen *et al.*, 2000; Braun-Kiewnick *et al.*, 2000; Liljeroth and Bryngelsson, 2002; Khan and Doohan, 2008a, b). Different substrates have been used in pot trials including vermiculite (Ali-Hoimoud *et al.*, 1993), sand (Jensen *et al.*, 2002) and soil (Jørgensen *et al.*, 1996). The more complex the pot trial system, the better it represents field conditions; thus it is preferable to use soil instead of vermiculite. However, when disease symptoms are evaluated on roots, it can ease the work flow not to use soil. At the less complex end of the spectrum, Yekkour *et al.* (2012) used Petri dishes with filter paper and four studies used tube assays with either vermiculite (Monfort *et al.*, 2005; Maciá-Vicente *et al.*, 2008, 2009) or cotton (Mostafa, 1993). Two studies used *in vitro* experiments of BCA and pathogen only (Ali-Hoimoud *et al.*, 1993; Moya *et al.*, 2016). Ali-Hoimoud *et al.* (1993) used a cut straw assay because they were interested in biocontrol of the survival structures of *Pyrenophora teres* on crop residues. The study by Moya *et al.* (2016) performed a 'classical' dual culture test using only one type of medium. This could be considered controversial because various studies have shown that type of media and water potential within the medium will influence growth rates, production of secondary metabolites and hyphal interactions between antagonist and pathogen (Whipps, 1987; Whipps and Magan, 1987).

Six of the eight endophyte studies checked whether their control agent could colonise barley as an endophyte (Vilich *et al.*, 1998; Waller *et al.*, 2005; Deshmukh and Kogel, 2007; Maciá-Vicente *et al.*, 2008, 2009 Achatz *et al.*, 2010). If the reduction in disease symptoms is linked to the lifestyle of the microorganism as an endophyte then it is relevant to show that the endophyte colonises the plant in question as an

Table 5.2 Summary of the best biological control results from the most complex systems obtained against five of the most commonly studied pathogens in barley.

The origin of the biological control organism is also listed. Names of organisms are given according to Species Fungorum (www.speciesfungorum.org).

Pathogen	Disease control (%)	Test system	Origin of BCA or endophyte	Author
Bipolaris sorokiniana	43	Field experiments	Soil	Knudsen *et al.*, 1995
Blumeria graminis f.sp. *hordei*	70	Pot experiment	Mammalian faeces	Choi *et al.*, 2007
Fusarium culmorum	73	Field experiment	Barley roots	Jensen *et al.*, 2000
Gaeumannomyces tritici	88	Tube with vermiculite	Endophyte of *Ammophila arenaria* ssp. *australis* (Poaceae)	Maciá-Vicente *et al.*, 2008
Pyrenophora teres	98	Field experiments	Roots of wild and cultivated plants	Hökeberg *et al.*, 1997

endophyte, especially, but not exclusively, if the endophyte has been sourced from a different species than the crop.

5.2.2 Biological Control Efficiency

Biological control efficiency varies among experiments with the best results for the most complex test systems summarised in Table 5.2. It is clear that barley diseases can be controlled using BCAs as well as endophytes in pot and in field trials. Beneficial microorganisms have been discovered from many and varying places and the best ones have originated from barley itself, marram grass (*Ammophila arenaria* ssp. *australis*), wild and cultivated plants and mammalian faeces (Table 5.2).

The experimental test system will influence the reported outcomes. The fewer the variables in the experiments, the easier it will be to obtain efficient biocontrol results. In the investigations, which tested biological control agents first in pot trials and later in the field, there was a tendency for the efficiency of the control agents to be 4–35% lower in the field (Knudsen *et al.*, 1995; Hökeberg *et al.*, 1997; Braun-Kiewnick *et al.*, 2000; Jensen *et al.*, 2000; Khan and Doohan, 2008a, 2008b). As an exception, Hökeberg *et al.* (1997) reported a specific *Pseudomonas* strain (MA 342), which controlled disease slightly better in the field (98%) compared to the pot trial (75%). If the treatment works in a pot experiment it will have a higher likelihood of success in the field than if the BCA was identified *in vitro*.

5.2.3 Biocontrol Mechanisms Used Against Barley Diseases

Ten studies (Table 5.1) have investigated the mechanisms behind the biological control, but rigorous evaluations are rare. In many cases, the potential involvement of all the possible mechanisms in biological control (antibiosis, competition, parasitism and induced resistance) have not been studied or even been possible to study simply because an appropriate experimental setup has not been applied. For example, to show that induced resistance is involved in biological control, requires plant experiments to be performed and defence responses to be studied.

Two of the studies used *Chaetomium* spp. endophytes as BCAs and only mechanisms inferred from *in vitro* assays were reported (Vilich *et al.*, 1998; Moya *et al.*, 2016). Both studies showed that *Chaetomium* spp. worked through antibiosis against leaf pathogens of barley *in vitro*. Vilich *et al.* (1998) concluded that their fungal isolate reduced spore germination of barley powdery mildew by antibiosis. They spread conidia of *Blumeria graminis* f. sp. *hordei* on malt extract agar plates that contained a filtrate of the BCA. However, they did not outline their control treatment, which makes it difficult to evaluate their findings and, furthermore, since the pathogen is an obligate biotroph, their *in vitro* setup may yield different results from a more realistic situation using barley leaves. In subsequent pot experiments, a BCA spore suspension was applied to the seeds and the pathogen was inoculated onto the leaves. It is, however, difficult to make firm conclusions on the mechanism *in planta* from the *in vitro* study. Thus, it is not known whether compounds of the endophyte reached the leaves, which would be a prerequisite for concluding that metabolites produced by the BCA was responsible for any disease reducing effect. Moya *et al.* (2016) performed a dual culture test where they placed a plug of the *Chaetomium* antagonist on a PDA plate and 3 days later placed a plug of either *Bipolaris sorokiniana* or *Pyrenophora teres* at a distance of 4 cm away from the first plug. The control treatment was the pathogen alone, which is perhaps not the optimal control as it may be argued that a proper control would have been a pure agar plug placed on a plate and a pathogen plug added 3 days later to exclude any effect of the agar. The conclusion was that the *Chaetomium globosum* isolates worked through antibiosis and competition against *Bipolaris sorokiniana* and through competition and mycoparasitism against *Pyrenophora teres.* These conclusions are all based on evaluations using a microscope and, unfortunately, these observations stand alone. Thus, it is unknown whether the endophytes had a similar behaviour *in planta* or whether they would be able to induce resistance against the pathogen.

Four studies investigated the mechanisms of control exerted by the endophyte *Serendipita indica* (Waller *et al.*, 2005; Deshmukh and Kogel, 2007; Achatz *et al.*, 2010; Harrach *et al.*, 2013). All studies used pathogens from the genus *Fusarium* and Waller *et al.* (2005) also included *Blumeria graminis* f. sp. *hordei* and *Bipolaris sorokiniana*, all in separate experiments. Harrach *et al.* (2013) and Waller *et al.* (2005) both concluded that elevated antioxidative capacity was the mechanism for disease

control, whereas Achatz *et al.* (2010) suggested that the endophyte used plant growth promotion to avoid disease and Deshmukh and Kogel (2007) concluded that pathogenesis-related (PR) proteins were not involved in protection. The main aim of the investigation by Achatz *et al.* (2010) was to show that *S. indica* relieved plants from nutrient stress and *Fusarium* sp. was used as an additional biotic stress. They showed that plants with and without *Fusarium* infection had equivalent grain yields. As grain yield is not a reliable measure of biological control and disease symptoms were not evaluated, it is difficult to discern if the pathogen was established and one must therefore be cautious in interpreting the results. Harrach *et al.* (2013) used *S. indica* against *F. culmorum* in a pot trial. No direct symptom scoring was made, but they used the shoot/root biomass as a proxy for disease scoring and they did quantify pathogen biomass as an indication of disease pressure. Antioxidant status of the roots was examined through ascorbate and glutathione levels as well as antioxidant enzyme activity. It was concluded that *S. indica* altered the antioxidant status of the cells so that they could detoxify excess reactive oxygen species (ROS) produced by the pathogen. However, in the literature used to indicate how the pathogen is affected by ROS, the authors only show 'plausible' correlations between *F. culmorum* and ROS production in *Arabidopsis* floral tissue. So, it is not entirely clear whether these responses can explain reductions in disease in barley.

The study of Waller *et al.* (2005) also used shoot/root biomass as an indicator of biological control for *F. culmorum* and the data for *B. sorokiniana* are not shown. It is suggested that the mechanism cannot be antibiosis because this was ruled out in a study in axenic culture, but data are not shown. Furthermore, it is not clear how plant inoculation with *S. indica* took place and, therefore, it is difficult to evaluate the relevance of the *in vitro* study. Antioxidant capacity was also studied when inoculating roots with and without *S. indica*. Since the pathogen was not present in these experiments, it is difficult to make conclusions about the mechanisms of control. Waller *et al.* (2005) also examined the control of *Blumeria graminis* f. sp. *hordei* and used a disease index to show reduction in disease symptoms in a detached-leaf assay, but again the antagonist delivery system is not clear. This time, systemic resistance was suggested.

Deshmukh and Kogel (2007) also ruled out antibiosis based on dual culture tests, although the nature of these experiments was not fully described. Perhaps it is too early to rule out antibiosis when there have been no additional tests of whether the endophyte can produce antagonistic compounds within the plant. The authors found that PR-protein genes were expressed at lower levels when *S. indica* was present with *F. culmorum* compared to plants inoculated with *F. culmorum* alone. They, therefore, concluded that PR proteins were not involved in the protection induced by the endophyte.

Khan *et al.* (2006) also examined the biocontrol of *Fusarium* spp., but they used bacteria as their control agents. Antibiosis was excluded as a potential mechanism

using *in vitro* inhibition zone studies on one type of medium. Again, it is perhaps premature to completely rule out antibiosis because of the absence of an inhibition zone when it is not clear what the BCA produces *in planta*. Their subsequent *in planta* study was only conducted on wheat and the results indicate that induced resistance is the mechanism involved. They examined the expression of a PR-gene (class III peroxidase), which is known to be upregulated in wheat in response to *Fusarium* infection. In this experiment, they worked with *Pseudomonas fluorescens* (MKB 156) and *Pseudomonas* sp. (MKB 158) and they were only able to show induced resistance for one of the strains (MKB 158).

The three last studies (Table 5.1) concern control of *Bipolaris sorokiniana* (Liljeroth and Bryngelsson, 2002; Jensen *et al.*, 2016) and/or *Pyrenophora teres* (Jørgensen *et al.*, 1998; Jensen *et al.*, 2016). Furthermore, Jensen *et al.* (2016) also included *Blumeria graminis* f. sp. *hordei* and *Rhynchosporium graminicola*. All three investigations used different BCAs. Thus, Jørgensen *et al.* (1998) used two non-barley pathogens to control diseases, whereas Liljeroth and Bryngelsson (2002) used *Microdochium bolleyi* as a BCA and Jensen *et al.* (2016) used *Clonostachys rosea*. Jørgensen *et al.* (1998) found that induced resistance was probably the main mechanism involved in the local protection exerted by the two non-barley pathogens. They showed that appressoria-formation was reduced and that papillae formation was increased. In the study by Jensen *et al.* (2016), *C. rosea* was able to control *Bipolaris sorokiniana*, *P. teres* and *R. graminicola*, but mechanisms of control were only evaluated for *B. sorokiniana*. It was concluded that the inhibition was direct and therefore probably involved mycoparasitism, competition and/or antibiosis. This was based on the fact that germination of pathogen conidia and inhibition of appressorial formation was observed. Induced resistance was ruled out because expression of three PR-protein genes was not increased in plants treated with antagonist and pathogen compared to the control, and furthermore, there was no increase in defence responses when evaluated under the microscope. In the study by Liljeroth and Bryngelsson (2002), *B. sorokiniana* was suggested to be controlled by induced resistance and this was shown by the elevated expression of PR-protein genes. However, results for pathogen and antagonist treated plants were performed separately and there was no treatment with both pathogen and antagonist together so it is not clear whether the antagonist can upregulate PR-protein genes in the presence of the pathogen.

5.3 Conclusion

There is a need to address the heavy reliance on agrochemicals in barley production and improve the environmental sustainability of the industry. Integrated pest management is encouraged within the European Union and biological control can be

incorporated into this approach. It is evident from the literature review conducted here that living microorganisms can control barley diseases in controlled laboratory experiments and, more importantly, under field conditions. It is furthermore clear that some of the most problematic diseases of barley in Northern Europe, including rusts, Ramularia leaf spot and barley yellow dwarf have not been challenged using biological control in agricultural systems. There is a trend in legislation for restricting the use of certain agrochemicals and organic agriculture is increasing globally each year which means that there will be a huge demand for non-chemical control methods for these diseases in the future.

Another finding is that there is no particular, specific niche from where to isolate biocontrol antagonists. It appears that it is possible to find antagonists in many types of environments. However, the majority of studies sourced their control agents from barley plants, other cereals or wild grasses and some of the best results were also obtained with BCAs obtained from such hosts. Endophytes also showed good results and they were sourced from barley leaves, wild grasses and from unrelated plant species. There is very little known about the host range of endophytes and it seems theoretically more likely that reliable results will be obtained when looking for endophytes from the crop of interest or its wild relatives because the chance of successful establishment within the plant is increased. Also, work with endophytes is recommended because endophytes can be protected within the plant and also have a biocontrol potential for multiple diseases.

Very few investigations have examined the mechanisms behind the biological control reported in barley, and within these, rigorous investigations were found to be infrequent. There is a need for the biocontrol research community to agree on standards in order to conclusively demonstrate biological control and determine the mechanisms involved. Appropriate disease symptoms must be evaluated and it is essential to choose relevant control treatments. Furthermore, gene expression studies or other studies to quantify defence responses in plants need to include treatments with pathogen and antagonist present together to compare with treatments with pathogen alone and quantify defence responses with a documented effect against the pathogen in question. When using endophytes to control diseases, it is also important to show that the endophyte can establish within the plant.

The trend in biological control research is to isolate control agents that can reduce symptoms from more than one disease or combine control agents in synergistic consortia. Such BCAs should have different modes of action. Ideally, a control agent should also be found which controls the pathogens in such a way that the pathogen does not evolve quickly to overcome the mechanism. Induced resistance is one such example because it generally elicits multiple defence reactions in the plant and thereby becomes difficult to overcome. Conversely, antibiosis might not be the best approach for biocontrol in barley and other crops because the pathogen

population might develop tolerance to the active compound, as they are known to do with agrochemicals.

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